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Assessing the effects of seawater temperature and pH on the bioaccumulation of emerging chemical contaminants in marine bivalves

Ana Luísa Maulvault[a,b,c,*], Carolina Camacho[a], Vera Barbosa[a], Ricardo Alves[a], Patrícia Anacleto[a,b,c], Fabiola Fogaça[d], Christiaan Kwadijk[e], Michiel Kotterman[e], Sara C. Cunha[f], José O. Fernandes[f], Rie R. Rasmussen[g], Jens J. Sloth[g], Òscar Aznar-Alemany[h], Ethel Eljarrat[h], Damià Barceló[h], António Marques[a]

[a] Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Lisbon, Portugal
[b] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal
[c] MARE – Marine and Environmental Sciences Centre, Faculty of Sciences, University of Lisbon (FCUL), Lisboa, Portugal
[d] EMBRAPA, Embrapa Meio-Norte, Parnaíba, Brazil
[e] Wageningen University & Research, AB Ijmuiden, The Netherlands
[f] LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[g] National Food Institute, Technical University of Denmark, Søborg, Denmark
[h] Water and Soil Quality Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, (IDAEA-CSIC), Barcelona, Spain

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ABSTRACT

Emerging chemical contaminants [e.g. toxic metals speciation, flame retardants (FRs) and perfluorinated compounds (PFCs), among others], that have not been historically recognized as pollutants nor their toxicological hazards, are increasingly more present in the marine environment. Furthermore, the effects of environmental conditions (e.g. temperature and pH) on bioaccumulation and elimination mechanisms of these emerging contaminants in marine biota have been poorly studied until now. In this context, the aim of this study was to assess, for the first time, the effect of warmer seawater temperatures ($\Delta = +4$ °C) and lower pH levels ($\Delta = -0.4$ pH units), acting alone or combined, on the bioaccumulation and elimination of emerging FRs (dechloranes 602, 603 and 604, and TBBPA), inorganic arsenic (iAs), and PFCs (PFOA and PFOS) in two estuarine bivalve species (*Mytilus galloprovincialis* and *Ruditapes philippinarum*). Overall, results showed that warming alone or combined with acidification promoted the bioaccumulation of some compounds (i.e. dechloranes 602, 604, TBBPA), but also facilitated the elimination of others (i.e. iAs, TBBPA). Similarly, lower pH also resulted in higher levels of dechloranes, as well as enhanced iAs, PFOA and PFOS elimination. Data also suggests that, when both abiotic stressors are combined, bivalves' capacity to accumulate contaminants may be time-dependent, considering significantly drastic increase observed with Dec 602 and TBBPA, during the last 10 days of exposure, when compared to reference conditions. Such changes in contaminants' bioaccumulation/elimination patterns also suggest a potential increase of human health risks of some compounds, if the climate continues changing as forecasted. Therefore, this first study pointed out the urgent need for further research on the effects of abiotic conditions on emerging contaminants kinetics, to adequately estimate the potential toxicological hazards associated to these compounds and develop recommendations/regulations for their presence in seafood, considering the prevailing environmental conditions expected in tomorrow's ocean.

1. Introduction

The remarkable increase of the human footprint on the planet (i.e. world population constant growth, excessive use of natural resources, and massive production of pollutants), especially since the mid-20th century, has contributed to one of the greatest environmental concerns of our time: climate change. Climate change effects can already be felt in many regions of the world, and are expected to worsen in the coming 50–100 years, with devastating consequences at ecological and human scales, even if strong efforts are made to maintain greenhouse gas

* Correspondence to: Portuguese Institute for the Sea and Atmosphere, I.P. (IPMA), Lisboa, Portugal.
E-mail address: aluisa@ipma.pt (A.L. Maulvault).

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emissions (GHGs) at the current levels (IPCC, 2014). Such changes also affect marine ecosystems, leading to a potential increase of the average seawater surface temperature (up to + 4 °C), as well as to ocean acidification due to increased levels of atmospheric CO_2, which dissolves into the ocean, dropping seawater pH down to 0.4 units in some areas of the globe (according to scenario 8.5 of the Representative Concentration Pathways of GHG concentrations, i.e. RCP 8.5, of the Intergovernmental Panel for Climate Change, IPCC, 2014). Depending on the region, both climate change effects can act independently as a single stressor, promoting deleterious alterations in marine species metabolism, growth, reproduction, among others, or can occur simultaneously (i.e. combined with each other or with other climate stressors, e.g. hypoxia, salinity) representing additional challenges to the resilience of marine ecosystems (e.g. Rosa et al., 2016; Maulvault et al., 2016, 2017; Sampaio et al., 2017).

Marine species are currently chronically surrounded by an array of chemical contaminants, particularly those inhabiting areas that are more vulnerable to anthropogenic impacts (e.g. estuaries and coastal lagoons) (e.g. Bollmann et al., 2012; Maulvault et al., 2015). New chemical substances that have not been historically recognized as pollutants, and for which limited toxicological information is still currently available, are increasingly more present in the marine environment (e.g. Feo et al., 2012; Cunha et al., 2015; Marques et al., 2015; Van der Meersch et al., 2015). These "contaminants of emerging concern" include various compounds from distinct chemical groups, which may occur naturally in the environment [e.g. inorganic arsenic (iAs)] or are exclusively man-made substances [e.g. flame retardants (FRs), perfluorinated compounds (PFCs), pharmaceutical residues, UV-filters and musks, among others] commonly derived from domestic, hospital and industrial effluents, as well as, agriculture and aquaculture activities (e.g. Bollmann et al., 2012; Feo et al., 2012; Maulvault et al., 2015; Van der Meersch et al., 2015). Arsenic is an ubiquitous element that can occur in the aquatic environment in several oxidation states (− 3, 0, + 3 and + 5), although being mostly found in its most toxic forms, i.e. the inorganic ones (AsIII or AsV; Matschullat, 2000). Arsenic concentrations in coastal ecosystems may range from 1 to 20 μg L^{-1} (Smedley and Kinniburgh, 2001), and in biota the highest concentrations are usually found in bivalve species (total As concentrations up to 24 μg g^{-1} dry weight (dw) (Sloth and Julshamn, 2008; Maulvault et al., 2015). Flame retardants (FRs) are persistent contaminants in the environment, particularly accumulating in sediments and biota, since most of them are extremely hydrophobic. Thus, their concentrations in seawater are usually undetectable or within the range of pg L^{-1} (e.g. Bollmann et al., 2012), whereas in marine sediments and biota values are in the order of pg g^{-1} dw and ng g^{-1} lipid weight (lw), respectively (e.g. Feo et al., 2012; Santín et al., 2013; Van der Meersch et al., 2015). Tetrabromobisphenol A (TBBPA) is one of the most relevant FR widely used by the industry and can be found in river and estuarine sediments, as well as in biota, reaching up to 14 ng g^{-1} of lw (EFSA, 2011; Van der Meersch et al., 2015). As for other types of FRs, based on their known toxic effects, recently, the EU banned or restricted the use of certain compounds (e.g. PBDEs, Mirex), which were replaced by new substances, defined as emerging FRs [e.g. dechloranes (Decs); Feo et al., 2012] for which limited information is available about their levels in marine environments. PFCs (e.g. perfluorooctanesulfonic acid, PFOS, and perfluorooctanoic acid, PFOA) are molecules composed by carbon chains strongly bound to fluorine atoms, which are widely used in various industrial and consumer activities (e.g. stain-resistant coatings for fabrics and carpets, fire-fighting foams and floor polishes, among others). Their strong carbon:fluorine bounds make them extremely resistant to degradation and, therefore, persistent over time in biological compartments. In aquatic environments, PFCs' concentrations can vary from undetectable to values surpassing 100 ng L^{-1} (Flores et al., 2013) and even exceeding 500 ng g^{-1} of wet weight (ww) in aquatic biota (e.g. EFSA, 2008; Van der Meersch et al., 2015).

Chemical contaminants' availability in marine sediments/water column and toxicity to biota are strongly influenced by environmental drivers, such as temperature, pH, salinity, upwelling and stratification events. Recent literature has intensively described climate change to likely have a direct impact on contaminants' physical-chemical properties and their partitioning among biological compartments (e.g. Noyes et al., 2009; Marques et al., 2010). Moreover, by altering species physiological status and, at the same time, exacerbating many forms of water pollution, climate change can promote deleterious impacts on marine organisms' physiology, hindering them to cope with the presence of chemical contaminants in the same way as they did before (e.g. Marques et al., 2010; Maulvault et al., 2016, 2017; Sampaio et al., 2017). On the other hand, changes in contaminants' uptake, retention and detoxification rates due to climate change may certainly compromise seafood safety, thus rising concerns from the public health point of view (e.g. Marques et al., 2010; Maulvault et al., 2016). Yet, given the limited number of empirical studies, with most available information being based on mechanistic approaches, the interaction between climate change and pollution still requires further understanding.

In this context, the aim of this study was to assess, for the first time, the effect of temperature (Δ = +4 °C) and pH (Δ = − 0.4 pH units), acting alone or in combination, on the bioaccumulation and elimination of emerging chemical contaminants (Dec 602, Dec 603, Dec 604, iAs, TBBPA, PFOA and PFOS), using estuarine bivalve species (*Mytilus galloprovincialis* and *Ruditapes philippinarum*) as biological models. The selection of the model species was based on the fact that these species can be considered suitable bioindicators of environmental pollution, since: i) they are filter-feeding and benthic organisms, thus potentially accumulating high levels of chemical contaminants, either dissolved or in suspended particulate forms, often reaching concentrations several orders of magnitude above the surrounding levels (e.g. Maulvault et al., 2015); and ii) they are among the most commercially important seafood species (both wild or farmed), with high consumption frequencies in Europe, thus enabling to establish a link with seafood safety.

2. Materials and methods

2.1. Experimental design

2.1.1. Chemical contaminants

The contaminants used in each exposure trial were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada) in the case of Dec 602 ($C_{14}H_4Cl_{12}O$, ≥ 98%), Dec 603 ($C_{17}H_8Cl_{12}$, ≥ 98%) and Dec 604 ($C_{13}H_4Br_4Cl_6$, ≥ 98%), or Sigma-Aldrich (USA) in the case of TBBPA ($((CH_3)_2C[C_6H_2(Br)_2OH]_2)$, 97%), iAs ($H_3AsO_4$, i.e. As(v) oxide, > 95%), PFOS (heptadecafluorooctanesulfonic acid solution, $CF_3(CF_2)_7SO_3H$, 10 μg mL^{-1}) and PFOA ($CF_3(CF_2)_6COOH$, 96%). The remaining reagents used to perform the quantification of each contaminant were of analytical grade or higher, and are given in Section 2.2 throughout the description of the respective methodologies, as well as in Supplementary materials.

2.1.2. Contaminant exposure

To perform contaminant exposure, two different approaches (i.e. exposure routes) were considered taking into account the specific physical-chemical properties of each selected compound: i) Trial I – Exposure via dietary sources through contaminant enriched feed. This exposure was carried out for compounds with hydrophobic behaviour, that are more commonly detected in marine sediments or can potentially be biomagnified along the food chain (due to their long half-lives in animal tissues). Thus, the compounds tested were Dec 602 (water solubility (WS) = 8.49 × 10^{-3} mg mL^{-1}), Dec 603 (WS = 3 × 10^{-2} mg mL^{-1}), Dec 604 (WS = 21 × 10^{-3} mg mL^{-1}; Feo et al., 2012) and TBBPA (WS = 6.3 × 10^{-5} mg mL^{-1}; EU, 2012); and ii) Trial II - Exposure via seawater spiked with contaminant solution. This exposure was carried out for compounds with high water solubility and which are commonly detected in seawater samples. Thus, the compounds

Table 1
Contaminant concentrations (mean ± standard deviation) in contaminated (exposure of Trial I) and non-contaminated (exposure of Trial II and elimination phase of Trials I and II) feeds, seawater (Trials I and II) and bivalve samples from CTR treatment (Trials I and II), as well as certified reference material values and limits of the detection (LOD) and quantification (LOQ) in each methodology used to quantify contaminants. Abbreviations: CTR – control (non-contaminated), 19 °C, 8.0 pH units; CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units; LW – lipid weight.

Contaminant	Nominal concentration (exposure)	Sampling day	Final concentration in seawater (μg L^{-1})					Final concentration in enriched feed	Contaminant concentration CTR bivalves	LOD		LOQ	
			CTR	CONT	CONT + Acid	CONT + Warm	CONT + Acid + Warm			Seawater samples	Bivalve samples	Seawater samples	Bivalve samples
Dec 602	50 ng g^{-1} (Feed)	Days 0–40	< LOD	< LOD	< LOD	< LOD	< LOD	10.0 ± 0.4 ng g^{-1}	< LOD (Trial I)	0.60 μg L^{-1}	21 pg g^{-1} lw	2 μg L^{-1}	70 pg g^{-1} lw
Dec 603	50 ng g^{-1} (Feed)	Days 0–40	< LOD	< LOD	< LOD	< LOD	< LOD	50.0 ± 2.0 ng g^{-1}	< LOD (Trial I)	0.10 μg L^{-1}	7 pg g^{-1} lw	0.3 μg L^{-1}	24 pg g^{-1} lw
Dec 604	50 ng g^{-1} (Feed)	Days 0–40	< LOD	< LOD	< LOD	< LOD	< LOD	1.0 ± 0.1 ng g^{-1}	< LOD (Trial I)	0.10 μg L^{-1}	7 pg g^{-1} lw	0.3 μg L^{-1}	24 pg g^{-1} lw
TBBPA	10 μg L^{-1} (Seawater)	Exposure (Days 2–20)	< LOD	9.6 ± 1.5	4.0 ± 1.3	9.6 ± 1.1	2.0 ± 1.1	–	< LOD (Trial II)	0.10 μg L^{-1}	0.3 μg kg^{-1}	1.0 μg kg^{-1}	0.25 μg kg^{-1}
		Elimination (Days 22–40)	< LOD	0.3 ± 0.5	< LOD	< LOD	< LOD						
iAs	100 μg L^{-1} (Seawater)	Exposure (Days 2–20)	74.6 ± 3.3	138.8 ± 9.3	145.1 ± 7.3	152.8 ± 13.6	145.6 ± 7.3	–	10.9 ± 4.3 ng - g^{-1} (Trial II)	0.30 μg L^{-1}	2.0 μg kg^{-1}	1.0 μg L^{-1}	4.0 μg kg^{-1}
		Elimination (Days 22–40)	71.2 ± 2.4	69.2 ± 6.7	75.1 ± 2.3	75.8 ± 4.6	72.3 ± 5.7						
PFOA	1 μg L^{-1} (Seawater)	Exposure (Days 2–20)	< LOD	1.0 ± 0.0	0.8 ± 0.3	1.1 ± 0.2	0.9 ± 0.2	–	< LOD (Trial II)	0.03 μg L^{-1}	0.30 μg L^{-1}	0.05 μg kg^{-1}	0.50 μg kg^{-1}
		Elimination (Days 22–40)	< LOD	< LOD	< LOD	< LOD	< LOD						
PFOA	1 μg L^{-1} (Seawater)	Exposure (Days 2–20)	< LOD	1.0 ± 0.0	1.8 ± 0.3	1.0 ± 0.1	1.1 ± 0.2	–	< LOD (Trial II)	0.03 μg L^{-1}	0.30 μg L^{-1}	0.05 μg kg^{-1}	0.50 μg kg^{-1}
		Elimination (Days 22–40)	< LOD	< LOD	< LOD	< LOD	< LOD						

tested were iAs (WS > 12 mg mL^{-1}; US National Research Council, 1977), PFOA (WS = 3.4 mg mL^{-1}; EFSA, 2008) and PFOS (WS = 0.52 mg mL^{-1}; EFSA, 2008).

As shown in Table 1, TBBPA was not successfully incorporated, using the available feed production methodology nor detected in the experimental feed bioaccumulation. Thus, since TBBPA bioaccumulation could not be studied using the first experimental approach (exposure via contaminant enriched feed), the second experimental approach (i.e. exposure via contaminated seawater) was used instead to study this compound.

For the first approach (i.e. exposure via contaminant enriched feed), bivalve powder feeds (particle size ~ 40 μm) were manufactured by SPAROS Lda (Olhão, Portugal). Briefly, ingredients were blended in a paddle mixer (Mainca RM90, Spain), micropulverized in a hammer mill (Hosokawa Micron, SH1, The Netherlands) and sieved below 25 μm. This feed was composed by 72.4% crude protein and 12.1% crude fat (full composition of feed can be consulted in Supplementary materials_Table 1). Contaminant stock solutions were then prepared, by solubilising an amount of each contaminant in < 5 mL chloroform (96%, Merck, USA), in order to achieve the nominal concentrations presented in Table 1 (i.e. ~ 100× the average concentrations found in marine sediments of contaminated coastal areas, to assure a clear bioaccumulation/elimination pattern was obtained during the selected experimental time; e.g. Feo et al., 2012; Sühring et al., 2015). Contaminant stock solutions were diluted in deionized water (total volume of 25 mL) and these solutions were top-sprayed on batches of the powder feeds with a pressurized spraying container (standard flat-fan nozzle; size 10 μm; pressure 2.7 bar). Upon coating the contaminant enriched feeds were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom) for 20 min at 40 °C. After feed preparation, contaminants' final concentration was then determined according to the methodologies described below (see Section 2.2; Table 1). A non-contaminated feed (maintenance feed to be used during the elimination phase of Trial I and during the whole Trial II) with the same composition was also prepared, following the same preparation protocol previously described, but without including the contaminant stock solutions, to maintain bivalves from control treatments (CTR) in both trials (I and II).

For the second approach (i.e. exposure via contaminated seawater; Trial II), contaminants stock solutions were prepared, by first solubilising an amount of each contaminant in < 5 mL of solvent (methanol, chloroform, or acid nitric, according to contaminant's chemical properties). All solvents were purchased from Merck (USA), and were of liquid chromatography gradient grade (methanol and chloroform) or supra pure (nitric acid 65%). The final volume of each stock solution was then adjusted with seawater to 500 mL (target nominal concentration shown in Table 1; nominal concentrations of ~ 10× the mean concentrations found in seawater samples from contaminated coastal areas were prepared, except for TBBPA for which 10 μg L^{-1} were used, to assure a clear bioaccumulation/elimination pattern was obtained during the experiment; e.g. Smedley and Kinniburgh, 2001, Flores et al., 2013).

2.1.3. Animal collection and acclimation

Bivalve specimens (Trial I: Japanese carpet shell clam, *Ruditapes philippinarum*, *n* = 1100, collection date – May 2015; Trial II: Mediterranean mussel, *Mytilus galloprovincialis*, *n* = 1100, collection date – March 2016) with similar dimensions were collected from the Tagus estuary (Lisbon, Portugal) and transported in appropriate refrigerated boxes (4 °C) to Guia Marine Laboratory (Faculty of Sciences, University of Lisbon, Portugal). Once at the laboratory, bivalves were randomly and equitably distributed in different rectangular shaped glass tanks (70 L of total capacity; 3 tanks per treatment) of a recirculation aquaculture system (RAS). Each tank had independent functioning. Temperature was set and adjusted whenever needed using an automatic seawater refrigeration system (± 0.1 °C; Frimar, Fernando Ribeiro Lda, Portugal), as well as, submerged digital thermostats (200 W, V2Therm, TMCIberia, Portugal). Seawater pH was set and maintained thanks to individual pH probes (GHL, Germany) connected to a computerized pH control system (± 0.1 pH units; Profilux 3.1 N, GHL, Germany), which monitored seawater pH in each tank every 2 s, and adjusted them whenever needed, via submerged air stones, by injecting CO_2 (Air Liquide, Portugal; to decrease pH) or by CO_2-filtered aeration (to increase pH) using air pumps (Stella 200, Aqua One Pro, Aqua Pacific UK Ltd, United Kingdom). Furthermore, each tank was equipped with independent UV disinfection (Vecton 300, TMC Iberia, Portugal) and biological filtration (FSBF 1500, TMC Iberia, Portugal) to maintain seawater quality parameters.

Bivalves were acclimated for a time period of 7 days, at the following conditions: dissolved oxygen > 5 mg L^{-1}; temperature = 19 ± 0.5 °C, pH = 8.00 ± 0.05 units, salinity = 35 ± 1% and photoperiod of 12 h light and 12 h dark (12L:12D). Ammonia (NH_3/NH^{4+}), nitrite (NO^{2-}) and nitrate (NO^{3-}) concentrations were daily checked (Tropic Marin, USA), and kept below detectable levels (i.e. < 0.02 mg L^{-1}), with the exception of nitrates, which were kept below 2.0 mg L^{-1}. Seawater total alkalinity was also measured in every tank on a weekly basis, following a protocol previously described (Sarazin et al., 1999) and the combination of total alkalinity (AT) and pH was used to calculate carbonate system parameters (average values obtained for each treatment can be consulted in Supplementary materials_Table 2). Bivalves were fed at least three times a day with non-contaminated feed (maintenance diet; 2% of the average animal body weight, bw). On a daily basis, animal condition was checked (i.e. dead animals were removed) and 25% of the total water volume in each incubation tank was exchanged.

Four days before initiating contaminants' exposure, seawater temperature and pH were slowly adjusted (+ 1 °C and − 0.1 pH units per day) in the corresponding tanks, until reaching the target values in tanks/treatments with higher seawater temperature and/or lower pH. The experimental setup (i.e. crossed treatments) used in both trials is schematized in Fig. 1, and comprised 5 treatments: i) CTR [(control; non-contaminated treatment to investigate possible external sources of contamination apart from feed (Trial I) or seawater (Trial II), in which specimens were maintained at reference temperature and pH conditions set according to the average values observed in Tagus estuary during summer (i.e. 19 °C and 8.0 pH units; Anacleto et al., 2014)], ii) CONT (contaminated and reference temperature and pH conditions]; iii) CONT + Acid (contaminated, reference temperature and pH set at 7.6 units, i.e. ΔpH = − 0.4 units according to scenario RPC8.5 of the IPCC, 2014); iv) CONT + Warm (contaminated, reference pH and temperature set at 23 °C, i.e. ΔTemperature = + 4 °C, according to scenario RPC8.5 of the IPCC, 2014); and v) CONT + Acid + Warm (contaminated, pH set at 7.6 units and temperature set at 23 °C). Each treatment was composed by three replicates (Fig. 1).

2.1.4. Contaminant exposure and elimination (Trials I and II)

In Trial I (i.e. exposure via contaminant enriched feed), *R. philippinarum* from each treatment were daily fed with the respective feeds (contaminated for treatments CONT and non-contaminated for treatment CTR; feed amount = 2% of the average animal bw, divided in at least 3 times a day) for a period of 20 days. Afterwards, an elimination phase was carried out for a similar period of time (i.e. another 20 days) by daily feeding animals from all treatments with non-contaminated feed.

In Trial II (i.e. exposure via contaminated seawater), each contaminated treatment was spiked at day 1 with the contaminant stock solutions in order to achieve the nominal concentrations targeted for each contaminant (Table 1), while *M. galloprovincialis* were daily fed with non-contaminated feed during the 20 days of exposure to contaminants (i.e. control feed; feed amount = 2% of the average animals body weight, bw, divided in at least 3 times a day). The target nominal concentrations, as well as, the final contaminant concentrations in each

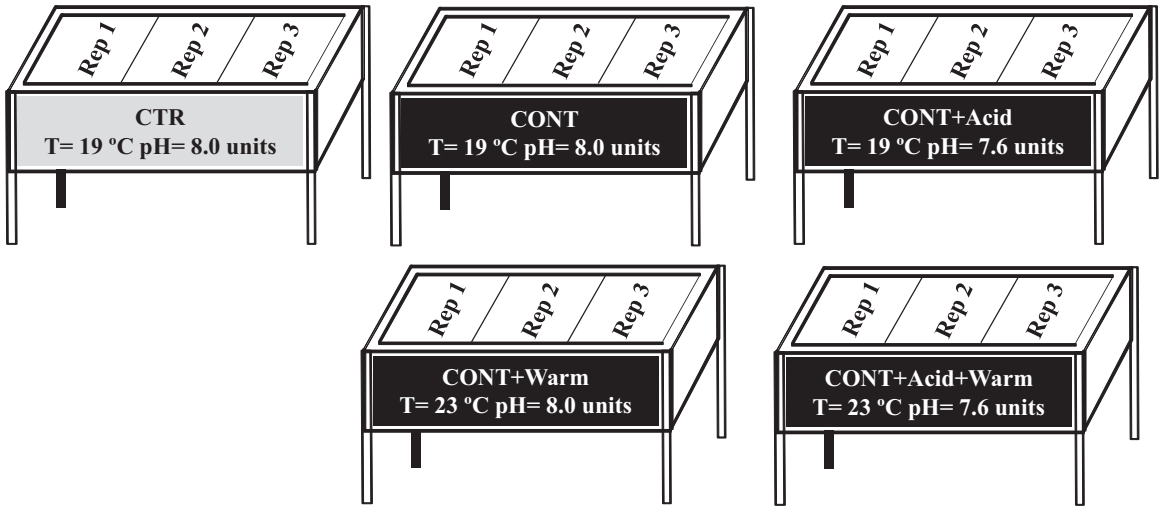

Fig. 1. Experimental setup in Trials I and II. Abbreviations: Rep – replicate of each treatment; CTR – control (non-contaminated), 19 °C, 8.0; CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units.

contaminated treatment are shown in Table 1. Control tanks (i.e. treatment CTR) were also spiked with equivalent amounts of solvent to ensure that no carrier solvent toxicity occurred. On a daily basis, seawater in each tank was partially replaced (~ 25%) and a volume of the contaminant stock solution (proportional to the 25% water replacement, thus, accounting for concentration lowering due to water exchange) in order to assure a steady contaminant concentration throughout the exposure phase. Afterwards, an elimination phase was carried out (daily contaminant spiking stopped) for 20 days.

In both trials, seawater physical-chemical parameters were daily checked and adjusted at optimum levels whenever needed as previously described. No mortality was observed during experimental trials I and II. Thirty animals (n = 10 per replicate treatment) were randomly sampled from each treatment on days 0, 2, 10, 20 (exposure), 22, 30 and 40 (elimination). Bivalves' biometric data, i.e. total length (L, cm), height (H, cm), width (W, cm) of the shell, total weight (TW, g) and edible weight (EW, g), were registered. Then, edible tissues were collected, pooled (i.e. n = 3 pools per treatment, per sampling day), immediately frozen at − 80 °C (for 24 h), freeze-dried at − 50 °C, 10^{-1} atm of vacuum pressure, for 48 h (Power Dry LL3000, Heto, Czech Republic) and kept at − 80 °C until contaminant quantification was performed. Seawater samples were also collected from each tank at each sampling day and kept at 4 °C until further analysis, in order to: i) investigate possible external sources of contamination (seawater from CTR treatments; Trials I and II) ii) assure that no contaminant leaching was occurring from the feed (Trial I); and iii) determine contaminants' final concentrations in seawater of each tank/replicate throughout the 40 days of trial (Trial II).

2.2. Quantification of emerging contaminants

2.2.1. Dechloranes 602, 603 and 604 (Decs)

Dechloranes were extracted from feed and bivalve samples using a previously optimized method (de la Cal et al., 2003; Labandeira et al., 2007). For the dechloranes extraction in seawater, samples were first spiked with an internal standard (13C-syn-DP), followed by an ultrasound assisted extraction using 2 mL of hexane (15 min sonication) and centrifugation (7 min, 3500 rpm). The hexane was transferred to a vial. The extract was then reconstituted with 40 µL of toluene for the instrumental analysis. Dechloranes' quantification in all samples was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer (Santa Clara, USA). The instrumental conditions and elution program were based on previous work (Barón et al., 2012). Further details regarding this methodology can be consulted in Supplementary materials.

2.2.2. Inorganic arsenic (iAs) and total arsenic (total As)

In seawater samples the total concentration of As was determined, whereas only the inorganic fraction was quantified in bivalve samples (i.e. iAs). The extraction of iAs in bivalve samples was performed as previously described in Rasmussen et al. (2012), and iAs was subsequently quantified following the standard method (EN 16802:2016) recently issued by European Committee for Standardization (CEN, 2016). As species were separated on anion exchange High Performance Liquid Chromatography (1100 HPLC Agilent Technologies, Waldbronn, Germany) and detected by inductively coupled plasma mass spectrometry (Agilent 7500ce ICP-MS, Santa Clara, USA) in no gas mode (As75). Total arsenic in seawater samples was determined by direct injection of samples diluted in nitric acid (2%, v/v) in ICP-MS in no gas mode (As75) with rhodium (Rh103) as internal standard. Further details on these two methodologies can be consulted in Supplementary materials.

2.2.3. Tetrabromobisphenol A (TBBPA)

TBBPA in bivalve and feed samples was extracted using the following procedure based on QuEChERS – LLE extraction, which was previously described in more detail (Cunha et al., 2017). Sample extracts were analysed by LC-MS/MS, i.e. a high-performance liquid chromatography (HPLC) system Waters Alliance 2695 (Waters, Milford, MA, USA) interfaced to a Quattro Micro triple quadrupole mass spectrometer (Waters, Manchester, UK). TBBPA was analysed in seawater by direct injection on LC-MS/MS with prior addition of 80 µL of $TBBPA_{13}C_{12}$ (IS, 1000 µg L^{-1}). The optimized MS/MS parameters for the target analytes are described in Cunha et al. (2017). Further details regarding this methodology can be consulted in Supplementary materials.

2.2.4. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)

PFOS and PFOA were extracted and analysed in both seawater and biota samples using the methods previously described in Kwadijk et al. (2010). Instrumental analysis was performed using a Thermo Finnigan (Waltham, MA) Surveyor Autosampler and an HPLC system coupled with a Thermo Finnigan LCQ advantage Ion-Trap MS instrument with electrospray (ESI-MS/MS). Further details regarding this methodology can be consulted in Supplementary materials.

2.3. Data analysis

Animal condition index (CI_t) was calculated, in ww basis, according to Maguire et al. (1999):

$$CI_t = \frac{TW_t}{L_t \times H_t \times W_t} \times 10,000$$

where, *t* is the sampling time in days, TW is the animal total weight (g), and L, H and W are the length, height and width of the shell (cm), respectively. Bivalves' growth rate (GR; mg of ww day^{-1}) was calculated as described by Santana et al. (2017):

$$GR_t = (TW_t - TW_{t0})/t$$

where, TW is the average total weight (g) at t days of trial. The net accumulation rate for each contaminant at each sampling day (NAR; ng g^{-1} of dw day^{-1}) was determined assuming that bivalves were exposed to steady conditions (i.e. continuous contaminant exposure, as well as seawater abiotic parameters) and using the following equation (Santana et al., 2017):

$$NAR_t = \frac{([cont_t] - [cont_{t0}])}{t}$$

where, [cont] is the average contaminant concentration in bivalve tissues (i.e. contaminant bioaccumulated) after t days of exposure.

The percentage of contaminant lost during the elimination phase of each trial, i.e. the elimination factor (EF; %) was calculated according to the following equation:

$$EF = 100 - \left(\frac{[cont_t]}{[cont_{t20}]} \times 100\right)$$

where, [cont] is the average contaminant concentration in bivalve tissue after t days of elimination and $[cont_{t20}]$ is the average contaminant by the end of the exposure phase, i.e. day 20 (Jebali et al., 2014). EF was considered to be 0 whenever $[cont_{t22,30\ and\ 40}]$ higher than $[cont_{t20}]$.

To perform statistical analysis, data were first tested for normality of distribution (Kolmogorov–Smirnov's test) and homogeneity of variance (Bartlett's test), and Log-transformed whenever necessary to comply with both assumptions of the ANOVA test. Then, two-way ANOVA test was used to check for the presence or absence of significant differences between contaminated treatments, with temperature (19 °C or 23 °C) and pH (8.0 units or 7.6 units) as variables. Post-hoc Tukey HSD test was subsequently carried out to identify such differences. Finally, potential correlations between CI, GR, NAR and EF were investigated by means of Pearson's correlation analysis. Statistical analyses were performed at a significance level of 0.05, using STATISTICA™ software (Version 7.0, StatSoft Inc., USA).

3. Results

3.1. Contaminant levels in seawater, feed and CTR bivalve samples (Trials I and II)

In Trial I, Decs 602, 603 and 604 were not detected in seawater samples (both CTR and CONT treatments with concentrations < LOD), neither in Japanese carpet shell clam samples (*R. philippinarum*) collected in CTR treatment (Table 1). As for the enriched feed, despite equivalent amounts of Decs 602, 603 and 604 were added in order to reach the same final concentration (~ 50 ng g^{-1}), a remarkable loss of Dec 602 and Dec 604 occurred during feed preparation (final concentrations of 10.0 ± 0.4 ng g^{-1} and 1.0 ± 0.1 ng g^{-1} for Dec 602 and Dec 604, respectively; Table 1). Such differences in Decs' concentrations were subsequently accounted for when analysing data. As previously described, TBBPA was not detected in the contaminant enriched feed, therefore, the bioaccumulation of this compound was subsequently assessed in Trial II.

In Trial II, none of the selected contaminants were detected in seawater samples collected in CTR treatment (i.e. TBBPA, PFOA and PFOS concentrations < LOD), except iAs (74.6 ± 3.3 µg L^{-1}; Table 1). Such results translated into detectable levels of iAs in CTR mussel samples (10.9 ± 4.3 ng g^{-1}; Table 1), which were subsequently taken into account when analysing data from CONT treatments. As for seawater samples from all CONT treatments, PFOA and PFOS concentrations were stable and around the nominal value set for these compounds. Conversely, iAs final concentrations were also steady by slightly above the nominal concentration defined for this element (around 150 µg L^{-1}), whereas lower TBBPA final concentrations were found in seawater samples collected from treatments exposed to acidification (i.e. CONT + Acid = 4.0 ± 1.3 µg L^{-1}; CONT + Acid + Warm = 2.0 ± 1.1 µg L^{-1}; Table 1).

3.2. Trial I – exposure to Dec 602, Dec 603 and Dec 604 via enriched feed

Fig. 2 presents the concentrations of Decs 602, 603 and 604 in Japanese carpet shell clams' meat during 40 days of Trial I. During contaminant exposure, detectable levels of these three compounds were found in all clam samples collected from CONT treatments, with Dec 603 showing, overall, higher concentrations than Dec 602 and Dec 604, regardless of seawater temperature and pH (Fig. 2). Overall, warmer temperatures significantly increased Dec's 602 concentration, straight

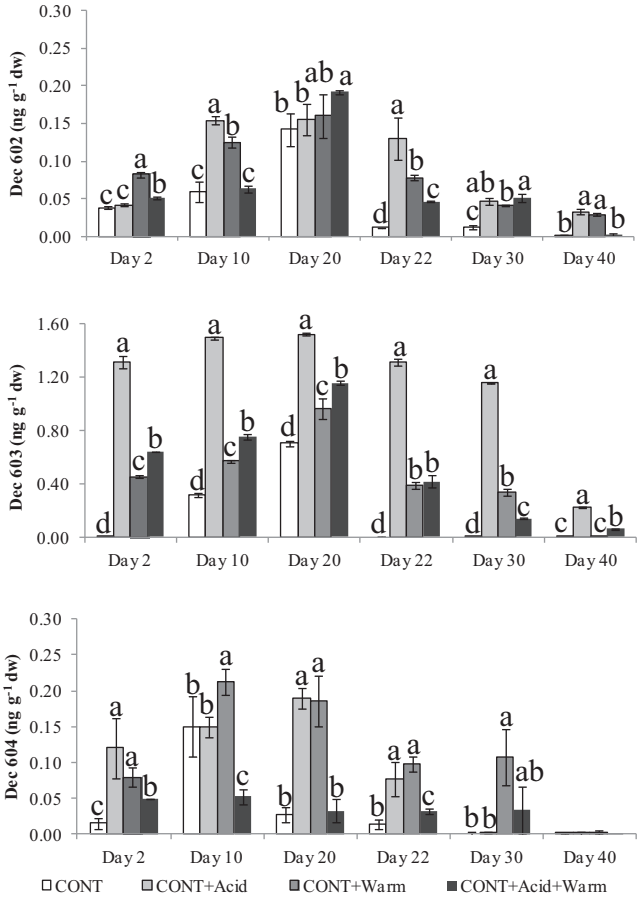

Fig. 2. Dechloranes' concentrations (dw) in Japanese carpet shell clam samples (*R. philippinarum*) from each contaminated treatment during the 20 days exposure and 20 days of elimination in Trial I (mean ± standard deviation; $n = 3$). Different letters indicate significant differences between treatments for each day ($p < 0.05$). Abbreviations: CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units.

from the second day of exposure ($p < 0.01$; Fig. 2 and Table 2). Furthermore, exhibiting a less pronounced increase in the first days of exposure compared the other treatments, after 20 days of exposure, treatment CONT + Acid + Warm registered the maximum Dec 602 concentration (i.e. 0.19 ng g^{-1} dw, equivalent to a NAR = 0.010 ng g^{-1} day^{-1}; $p < 0.01$; Fig. 2 and Table 2). Subsequently, a reduction was observed immediately after 2 days of depuration (i.e. day 22) in all treatments. Yet, acidification acting alone (i.e. CONT + Acid) resulted in the lowest EF for this compound after 20 days of elimination period (78%; $p < 0.05$; Table 2). As for Dec 603, lower pH levels, alone or combined with warmer temperatures, promoted significantly higher concentrations of this compound in clams' meat during the exposure phase (i.e. CONT + Acid = 1.53 ± 0.02 ng g^{-1} dw and CONT + Acid + Warm = 1.16 ± 0.01 ng g^{-1} dw), as well as during depuration (i.e. CONT + Acid = 0.23 ± 0.01 ng g^{-1} dw and CONT + Acid + Warm = 0.06 ng g^{-1} dw; $p < 0.01$; Fig. 2). Such trend resulted in significantly higher NARs and lower EFs in treatments CONT + Acid and CONT + Acid + Warm ($p < 0.05$; Table 2). Warming alone (i.e. CONT + Warm) has also lead to significant higher levels of Dec 603 at the end of the exposure and until day 30 compared to treatment CONT (Fig. 2; Table 2). Finally, during the 20 days of exposure, Dec 604 revealed significantly higher levels in bivalves subjected to warming and acidification acting alone (i.e. ~ 0.19 ng g^{-1} dw in CONT + Acid and CONT + Warm; $p < 0.01$), but not when both effects were combined (i.e. CONT + Acid + Warm; Fig. 2; Table 2). Despite the statistically higher concentrations of Dec 604 in the treatment CONT + Warm at days 22 and 30, by the end of the clearance period the percentages of elimination of this compound were similar among treatments (Table 2). Regarding animal condition (CI) and growth rate (GR; Table 3), although the CIs of contaminated animals were not significantly different from non-contaminated ones (i.e. CTR) nor within CONT treatments, overall, bivalves exposed to warmer temperatures revealed significantly higher GR than those exposed to the reference temperature or to low pH alone, namely at days 10 (CONT + Warm; $p < 0.05$), 20 (CONT + Warm and CONT + Acid + Warm; $p < 0.05$) and 30 (CONT + Acid + Warm; $p < 0.05$) (Table 3). Moreover, significant positive correlations were found between NAR of Decs 602, 603 and 604, and clams GR ($p < 0.05$; Table 4).

3.3. Trial II – exposure to TBBPA, iAs, PFOA and PFOS via contaminated seawater

As shown in Fig. 3, all mussel samples collected from CONT treatments revealed detectable levels of TBBPA, iAs, PFOA and PFOS. Concerning TBBPA, despite the significantly higher concentrations found in treatment CONT at day 10 of the trial, by the end of the exposure phase (day 20) mussels exposed to warming combined with acidification showed the highest concentrations (i.e. treatment CONT + Acid + Warm = 5716 ± 179 ng g^{-1} dw), as well as the highest NARs for this compound ($p < 0.05$; Fig. 3; Table 2). Furthermore, increased seawater temperature (with or without pH decrease) also led to a significantly higher elimination of TBBPA (~ 99% of TBBPA concentration at day 40 in these treatments; $p < 0.05$; Table 2). Conversely, significantly lower concentrations of TBBPA were found in mussels subjected to lower pH alone. As far as iAs and PFOA are concerned, warm and acid (alone or combined) treatments revealed significantly lower levels compared to those found in specimens exposed to reference temperature and pH conditions during exposure (CONT = 711 ± 3 ng g^{-1} dw and 20 ± 2 ng g^{-1} dw, for iAs and PFOA respectively, at day 20; $p < 0.05$; Fig. 3). As for the depuration period, iAs showed the highest EF in warming treatments (over 52% with or 71% without acidification; $p < 0.05$; Table 2), whereas acidification promoted a higher elimination of PFOA (90% with or 88% without warming; $p < 0.05$; Table 2). Finally, significantly lower PFOS concentrations were found in bivalves exposed to warming and acidification alone (i.e. CONT + Warm = 1476 ± 108 ng g^{-1} dw and CONT + Acid = 1910 ± 156 ng g^{-1} dw; $p < 0.01$; Fig. 3) compared to the CONT treatment (i.e. contaminated under reference temperature and pH), but not with the combination of both factors, whereas the highest percentages of elimination were found in CONT + Acid (99%; $p < 0.05$; Table 2), though not statistically different than mussels from the CONT treatment. Animal CI also did not vary significantly in Trial II, with the exception of day 30, in which bivalves exposed to warming (CONT + Warm) showed significantly higher CI than those exposed to acidification (CONT + Acid and CONT + Acid + Warm; $p < 0.05$; Table 3). Moreover, a significant negative correlation was found between CI and NAR of TBBPA and PFOS, but not for the remaining contaminants ($p < 0.05$; Table 4). In general, non-contaminated animals revealed significantly higher GR than contaminated ones exposed to the same temperature and pH values (i.e. CTR versus CONT; $p < 0.05$; Table 3). Yet, when exposed to warming alone or the combination of warming and lower pH, significantly higher mussels GR were observed compared to animals exposed to control temperature and pH (i.e. CONT), and to contaminated mussels under low pH during exposure (CONT + Acid). In contrast, by the end of the elimination period, treatments under reference temperature and pH (CTR and CONT) revealed statistically higher GR compared to the remaining treatments ($p < 0.05$; Table 3).

4. Discussion

Starting with contaminant levels in the enriched feed (Trial I), results evidenced that compound stabilization difficulties occurred during feed preparation for Dec 602 and Dec 604, as well as for TBBPA, which was also intended to be studied using the first experimental approach (exposure via enriched feed) in the first place, as previously mentioned. Such feed stabilization difficulties can be possibly due to contaminant adsorption to the equipments used during feed preparation and/or compound degradation (e.g. mechanic degradation or due to heat exposure). Furthermore, the considerable loss of TBBPA compared to Decs may be related to the fact that this compound is known to be less persistent and easily degraded, being subsequently transformed in BPA due to the loss of its bromine groups. As for contaminant levels in seawater samples, in Trial I results evidenced that: i) contaminant leaching from feed to seawater did not occur in Trial I; and ii) no other source of external chemical contamination apart from the enriched feed occurred.

Concerning seawater samples (Trial II), the levels of iAs found in CTR treatment (which translated in detectable concentrations of this compound in CTR mussels, as well as in iAs concentrations in CONT treatments above the defined nominal value) are most likely due to the presence of this compound in the maintenance feed used in these trials. On the other hand, the lower TBBPA concentrations observed in seawater samples from acid treatments (CONT + Acid and CONT + Acid + Warm) may be related to the fact that, as most ionisable compounds, TBBPA can alternate from molecular to ionic forms and vice-versa, precipitate or even be degraded according to the surrounding pH level.

Regarding bivalve samples from both trials, the higher concentrations of Dec 603 compared to Decs 602 and 604 found in clams' edible tissues (all CONT treatments) during the exposure phase can be partially attributed to the lower daily exposures of Dec 602 (~ 5 times) and even lower of Dec 604 (~ 50 times) compared to Dec 603, due to the considerable loss of these compounds during feed preparation (Table 1), as previously mentioned. It should be noted that differences in Decs' bioaccumulation can also be related to distinct bioavailability (i.e. readiness to be absorbed at an organism's digestive epithelia) of each compound, as it has been also reported in wild biota, with Dec 602 being often found in marine species at higher levels compared to other Dec's (i.e. Dec 603, Dec 604 and Dec Plus; Feo et al., 2012; Aznar-Alemany et al., 2016). The same fact can also justify the discrepancy in concentration ranges of PFOA a PFOS observed in Trial II, despite the similar concentrations in seawater, with the second exhibiting much

Table 2
Net accumulation rates (NAR; mean ± standard deviation) after 20 days of contaminant exposure and elimination factors (EF; mean ± standard deviation) after 20 days of elimination in contaminated treatments. In each column (and sampling day), different letters indicate significant differences between treatments ($p > 0.05$). Abbreviations: CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units.

Sampling day	Treatment	Dec 602		Dec 603		Dec 604	
		NAR ng g^{-1} dw day^{-1}	EF (%)	NAR ng g^{-1} dw day^{-1}	EF (%)	NAR ng g^{-1} dw day^{-1}	EF (%)
Day 20	**CONT**	0.007 ± 0.001^{b}	–	0.035 ± 0.001^{d}	–	0.001 ± 0.001^{b}	–
	CONT + Acid	0.007 ± 0.001^{b}	–	0.076 ± 0.001^{a}	–	0.010 ± 0.001^{a}	–
	CONT + Warm	0.008 ± 0.001^{ab}	–	0.048 ± 0.004^{c}	–	0.009 ± 0.002^{a}	–
	CONT + Acid + Warm	0.010 ± 0.000^{a}	–	0.058 ± 0.001^{b}	–	0.002 ± 0.001^{b}	–
Day 40	**CONT**	–	99.3 ± 0.9^{a}	–	99.9 ± 0.1^{a}	–	100.0 ± 0.0
	CONT + Acid	–	78.7 ± 5.2^{b}	–	85.2 ± 0.3^{c}	–	98.6 ± 2.0
	CONT + Warm	–	81.5 ± 4.2^{ab}	–	99.9 ± 0.2^{a}	–	99.0 ± 1.3
	CONT + Acid + Warm	–	99.2 ± 1.1^{a}	–	94.6 ± 0.2^{b}	–	99.7 ± 0.5

TBBPA		iAs		PFOA		PFOS	
NAR ng g^{-1} dw day^{-1}	EF (%)	NAR ng g^{-1} dw day^{-1}	EF (%)	NAR ng g^{-1} dw day^{-1}	EF (%)	NAR ng g^{-1} dw day^{-1}	EF (%)
243.4 ± 15.8^{b}	–	35.1 ± 0.2^{a}	–	0.98 ± 0.11^{a}	–	113.1 ± 2.7^{a}	–
150.9 ± 2.6^{c}	–	18.1 ± 0.6^{b}	–	0.45 ± 0.01^{b}	–	95.5 ± 7.8^{b}	–
256.5 ± 40.4^{ab}	–	17.6 ± 0.4^{b}	–	0.27 ± 0.07^{c}	–	73.8 ± 5.4^{c}	–
285.8 ± 9.0^{a}	–	7.3 ± 0.4^{c}	–	0.56 ± 0.12^{b}	–	112.6 ± 3.6^{a}	–
–	97.3 ± 0.3^{b}	–	42.2 ± 5.8^{c}	–	68.4 ± 3.5^{b}	–	97.8 ± 0.5^{ab}
–	96.1 ± 0.4^{b}	–	46.9 ± 1.5^{c}	–	88.3 ± 1.4^{a}	–	98.8 ± 0.1^{a}
–	99.7 ± 0.1^{a}	–	71.3 ± 0.9^{a}	–	73.7 ± 1.3^{b}	–	97.0 ± 0.5^{b}
–	99.5 ± 0.1^{a}	–	52.4 ± 0.4^{b}	–	90.5 ± 1.5^{a}	–	97.4 ± 0.4^{b}

Table 3
Bivalves' condition index (CI) and growth rate (GR) during 40 days of trial (20 days exposure + 20 days elimination; mean ± standard deviation). In each column (and day), different letters indicate significant differences between treatments ($p > 0.05$). Abbreviations: CTR – control (non-contaminated), 19 °C, 8.0 pH units; CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units.

		Trial I (*R. philippinarum*)		Trial II (*M. galloprovincialis*)	
		CI	GR (g day^{-1})	CI	GR (g day^{-1})
Day 0	**All**	6.92 ± 0.46	–	4.96 ± 0.66	–
Day 2	**CTR**	7.08 ± 0.48	1.06 ± 0.13	5.18 ± 0.28	0.59 ± 0.59^{ab}
	CONT	6.98 ± 0.48	0.90 ± 0.16	5.35 ± 0.16	0.25 ± 0.05^{b}
	CONT + Acid	7.30 ± 0.36	0.85 ± 0.11	5.13 ± 0.46	0.56 ± 0.18^{a}
	CONT + Warm	6.74 ± 0.36	1.34 ± 0.45	5.02 ± 0.54	0.63 ± 0.17^{a}
	CONT + Acid + Warm	7.52 ± 0.40	1.09 ± 0.25	5.11 ± 0.22	1.18 ± 0.77^{a}
Day 10	**CTR**	7.01 ± 0.33	0.18 ± 0.03^{b}	5.00 ± 0.41	0.50 ± 0.44
	CONT	6.84 ± 0.36	0.14 ± 0.01^{c}	5.23 ± 0.08	0.61 ± 0.36
	CONT + Acid	6.93 ± 0.53	0.13 ± 0.01^{c}	5.00 ± 0.67	0.83 ± 0.19
	CONT + Warm	7.54 ± 0.57	0.37 ± 0.03^{a}	5.21 ± 0.43	0.45 ± 0.33
	CONT + Acid + Warm	7.00 ± 1.16	0.23 ± 0.04^{b}	5.04 ± 1.67	0.71 ± 0.62
Day 20	**CTR**	7.11 ± 0.55	0.08 ± 0.01^{c}	5.25 ± 0.32	0.75 ± 0.18^{a}
	CONT	7.03 ± 0.32	0.09 ± 0.01^{bc}	5.14 ± 0.15	0.28 ± 0.11^{b}
	CONT + Acid	7.09 ± 0.51	0.06 ± 0.00^{d}	5.25 ± 0.23	0.11 ± 0.10^{b}
	CONT + Warm	6.96 ± 0.64	0.12 ± 0.01^{a}	5.25 ± 0.21	0.83 ± 0.12^{a}
	CONT + Acid + Warm	7.26 ± 0.27	0.11 ± 0.01^{a}	5.19 ± 0.53	0.79 ± 0.22^{a}
Day 22	**CTR**	7.23 ± 0.20	0.05 ± 0.01^{ab}	5.72 ± 0.72	1.43 ± 0.75^{a}
	CONT	7.32 ± 0.35	0.04 ± 0.01^{b}	5.62 ± 0.37	0.13 ± 0.12^{b}
	CONT + Acid	7.25 ± 1.04	0.08 ± 0.02^{a}	5.30 ± 0.24	1.19 ± 0.28^{b}
	CONT + Warm	7.26 ± 0.50	0.04 ± 0.01^{b}	5.63 ± 0.55	0.67 ± 0.54^{ab}
	CONT + Acid + Warm	7.46 ± 0.70	0.06 ± 0.01^{ab}	5.97 ± 0.90	0.82 ± 0.06^{ab}
Day 30	**CTR**	7.06 ± 0.43	0.03 ± 0.01^{ab}	5.73 ± 0.86^{ab}	1.01 ± 0.14^{a}
	CONT	7.13 ± 0.49	0.02 ± 0.01^{b}	5.68 ± 0.34^{ab}	0.19 ± 0.16^{b}
	CONT + Acid	7.28 ± 0.56	0.04 ± 0.00^{ab}	5.34 ± 0.14^{b}	1.22 ± 0.34^{a}
	CONT + Warm	7.12 ± 0.30	0.04 ± 0.02^{ab}	5.84 ± 0.17^{a}	0.33 ± 0.01^{b}
	CONT + Acid + Warm	7.11 ± 0.51	0.05 ± 0.01^{a}	5.37 ± 0.11^{b}	0.39 ± 0.22 ^{b}
Day 40	**CTR**	7.03 ± 0.53	0.01 ± 0.00	5.53 ± 0.26	0.72 ± 0.07^{a}
	CONT	7.10 ± 0.46	0.01 ± 0.01	5.40 ± 0.18	0.46 ± 0.01^{b}
	CONT + Acid	7.53 ± 0.35	0.02 ± 0.01	5.41 ± 0.28	0.19 ± 0.03^{c}
	CONT + Warm	7.57 ± 0.71	0.02 ± 0.01	5.62 ± 0.69	0.02 ± 0.00^{d}
	CONT + Acid + Warm	7.56 ± 1.31	0.02 ± 0.01	5.48 ± 0.24	0.01 ± 0.00^{d}

Table 4
Pearson's correlation coefficients between animal growth rate (GR), condition index (CI) and contaminants' net accumulation rates (NAR) and elimination factors (EF). Asterisks indicate significant correlations between variables ($p > 0.05$).

	r	
	NAR	**EF**
Trial I		
GR × CI	− 0.64	
GR × Dec 602	**0.90***	− 0.21
CI × Dec 602	− 0.24	− 0.04
GR × Dec 603	**0.66***	0.27
CI × Dec 603	0.12	− 0.23
GR × Dec 604	**0.77***	− 0.36
CI × Dec 604	− 0.02	0.03
Trial II		
GR × CI	− 0.25	
GR × TBBPA	0.17	− 0.14
CI × TBBPA	− **0.49***	− 0.02
GR × iAs	0.07	− 0.46
CI × iAs	− 0.35	0.11
GR × PFOA	0.04	− 0.27
CI × PFOA	− 0.36	0.10
GR × PFOS	0.25	0.19
CI × PFOS	− **0.43***	− 0.13

higher NAR during the exposure phase, regardless of the treatment, than the first one. In fact, the bioaccumulation of PFCs in biota, as well as their adsorption to sediments has been previously shown to be dependent on the length of the perfluorinated tail (e.g. Martin et al., 2004). A recent field study performed in marine biota from different taxonomic groups concluded that the bioaccumulation and elimination of these compounds is largely dependent on species and compound, with fish species showing, in general, higher levels of PFOS than PFOA, whereas bivalves showed the opposite trend, thus, contrasting the present findings (Hong et al., 2015).

As for the effects of temperature and pH, the current lack of empirical studies focusing on the bioaccumulation and toxicity of emerging contaminants, hinders adequate comparisons of the findings acquired in this first study with previous literature on contaminants' kinetics. Yet, the different trends observed in bivalves exposed to increased seawater temperature and reduced pH in this study highlighted the urgent need to consider the interactions between multiple stressors when assessing the potential environmental and human health risks of emerging contaminants' exposure, especially in the context of climate change.

By enhancing biota's metabolism, thus, increasing species ventilation and feeding rates in response to higher metabolic demands (Dijkstra et al., 2013), warmer temperatures can likely translate into higher contaminant bioaccumulation, as well as increased contaminant metabolization and excretion (e.g. Maulvault et al., 2016; Sampaio et al., 2016). Hence, such metabolic enhancement may justify the increased bioaccumulation of some compounds under warmer seawater temperatures, particularly those with long half-lives, such as Dec 602, Dec 603 and Dec 604, which are more likely to build up their concentrations in tissues under continuous exposure conditions due to their lipophilic behaviour and low elimination rates (Shen et al., 2010). Similarly, a recent study performed with juvenile seabass (*Dicentrarchus labrax*) exposed to methylmercury (MeHg) from dietary sources, also revealed higher MeHg bioaccumulation at warmer temperatures, as well as diminished ability to detoxify this persistent and biomagnifying pollutant (Maulvault et al., 2016). On the other hand, enhanced metabolic rates due to warming may translate into the opposite trend for compounds with lower ability to persist in biological compartments, i.e.

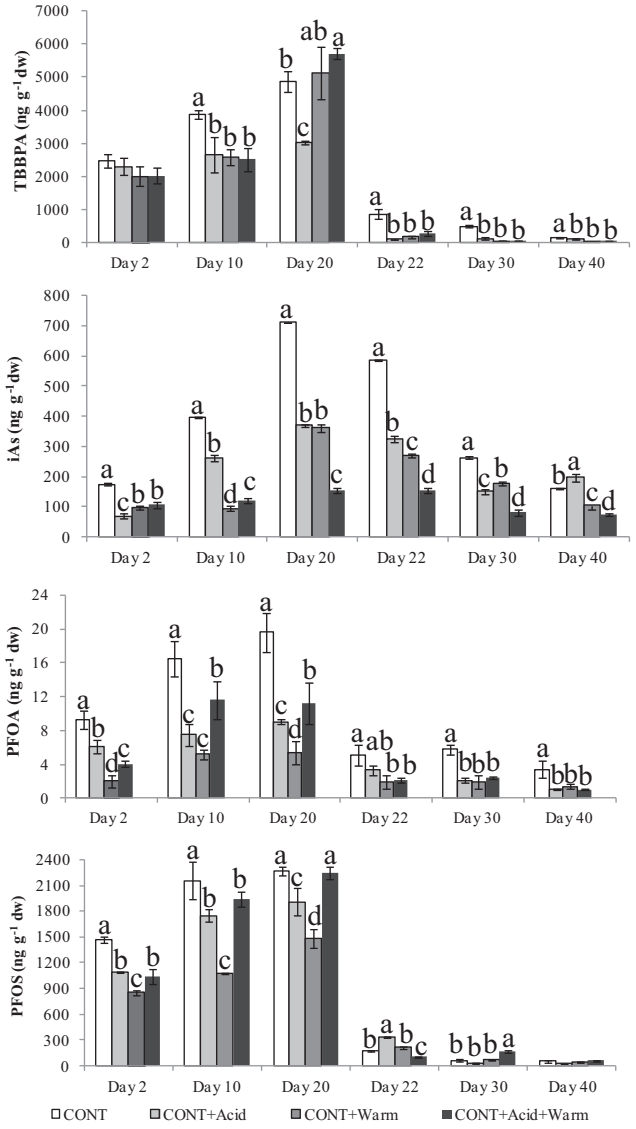

Fig. 3. Contaminants' concentrations (dw) in Mediterranean mussel samples (*M. galloprovincialis*) from each contaminated treatment during the 20 days exposure and 20 days of elimination in Trial II (mean ± standard deviation; $n = 3$). Different letters indicate significant differences between treatments for each day ($p < 0.05$). Abbreviations: CONT – contaminated, 19 °C, 8.0 pH units; CONT + Acid – contaminated, 19 °C, 7.6 pH units; CONT + Warm – contaminated, 23 °C, 8.0 pH units; CONT + Acid + Warm – contaminated, 23 °C, 7.6 pH units.

those with shorter half-lives, which can be transformed and subsequently excreted at faster rates (i.e. within 24–72 h; e.g. Vanden Heuvel et al., 1991; Knudsen et al., 2007; WHO, 2001), as observed in the present study for some compounds, such as iAs, TBBPA and PFOA. Furthermore, animal growth should also be accounted when interpreting contaminants' bioaccumulation in warmer environments, as increased feeding rates to support enhanced metabolic demands can also result in greater growth, leading to reduced contaminant bioaccumulation through somatic growth dilution (Dijkstra et al., 2013). Yet, despite few variations in GR, particularly in CONT + Warm and CONT + Warm + Acid treatments, the opposite trend was observed in terms of Decs' bioaccumulation (i.e. GR positively correlated with NARs), whereas there was no clear relation between GR and the bioaccumulation of the other compounds.

Concerning the effect of acidification, as observed in Decs 603 and 604, increased contaminant bioaccumulation has also been reported in some studies due to metabolic changes under hypercapnia (e.g. Rosa et al., 2016; Sampaio et al., 2016, 2017), as well as, damages in tissues' apical epithelial membrane that facilitate contaminant penetration into cells (Freitas et al., 2016; Sampaio et al., 2016, 2017; Shi et al., 2016; Velez et al., 2016). On the other hand, lower contaminant elimination at reduced pH levels has also been previously described due to the fact that bivalves possess a valve closing strategy of defence when exposed to stressors (i.e. surrounding pH outside species' optimal range; and/or contaminants exposure), thus, preventing the uptake of contaminants, as well as the excretion of compounds and their metabolites into the environment (Freitas et al., 2016; Velez et al., 2016). Such argument may justify the lower elimination of Dec 602, Dec 603, TBBPA and iAs under acid conditions. Yet, such physiological responses under reduced pH conditions seem to be reversed when warming is also added to the equation. Also worth mentioning, particularly in what concerns metals and other ionisable compounds, is the fact that the surrounding seawater pH levels strongly influences the chemistry, speciation and, thus, the availability of these compounds (e.g. Shi et al., 2016; Velez et al., 2016). Contrasting the results observed in the present study, Velez et al. (2016) reported an increase in iAs bioaccumulation at lower pH, justifying their results with the fact that the uptake of this element occurs via the phosphate transport systems (such as Na^+ and K^+-ATPase) which are also involved in pH osmoregulation of estuarine biota, thus probably leading to the competition for the same transport mechanisms (Monserrat et al., 2007). Yet, possible justifications for such differences in iAs bioaccumulation patterns include: i) different iAs forms used in each study (H_3AsO_4 in this study; $Na_2HAs_4^-$ in Velez et al., 2016); ii) iAs bioaccumulation depends on the tested pH level (8.0 and 7.6 pH units in the present study and 7.8 and 7.3 pH units in Velez et al., 2016); iii) iAs bioaccumulation may be a species dependent mechanism (*M. galloprovincialis* in the present study and *R. philippinarum* in Velez et al., 2016), given distinct filtration rates of bivalve species, as well as the ability for some species to keep valves closed as a strategy of protection against the exposure to contaminants and/or low pH (Freitas et al., 2016; Velez et al., 2016). As for the bioaccumulation and elimination of PFOA and PFOS, which was also affected by pH level, studies involving biota are extremely scarce and, to the authors' best knowledge, the present report is the first to explore the effect of pH on the bioaccumulation of these contaminants. Hence, despite it is not possible to compare the present data with previous reports on marine biota, both of these PFCs have been pointed out intensively in the literature to alter from molecular forms into ionic dissolved forms according to the surrounding pH conditions, with higher pH facilitating the uptake of adsorbed molecular forms (e.g. Higgins and Luthy, 2006; Wang et al., 2012). Furthermore, unlike other pollutants, PFOA and PFOS do not primarily accumulate in adipose tissues, but rather bind to proteins, such as albumin, which are mainly present in blood, liver and eggs (e.g. Martin et al., 2003). Since albumin is involved in organisms' osmoregulation, being responsible for controlling the osmotic pressure in tissues, changes in surrounding pH may interfere with albumin ability to bind to PFOS and PFOA, thus, resulting in lower tissue bioaccumulation. This pattern has been described in several pharmacokinetic studies involving vertebrate species and different chemical compounds (Kim et al., 1999; Hinderling and Hartmann, 2005). Regarding the combination of warmer temperature and lower pH, the distinct patterns compared to those observed when both stressors acted in isolation (e.g. after 20 days of exposure, Dec 604 and iAs: CONT + Acid and CONT + Warm significantly higher than CONT + Warm + Acid; PFOA and PFOS: CONT + Acid and CONT + Warm significantly lower than CONT + Warm + Acid), emphasized the importance of considering the interactions between different abiotic stressors in studies focused on contaminants' bioaccumulation kinetics. Particularly concerning TBBPA, the bioaccumulation trends observed for this compound (i.e. increased in CONT + Acid + Warm during the last 10 days of exposure) suggest that, though bivalves inhabiting estuarine, intertidal, and subtidal areas, like R. *philippinarum* and *M. galloprovincialis*, have developed strategies to cope with the presence of multiple

environmental stressors at the same time (i.e. pollution and wide ranges of abiotic conditions; Lannig et al., 2010), such physiological plasticity may: i) be time-limited, with species resilience to stress being committed under long-term and continuous exposure conditions (Belivermis et al., 2016); ii) have pushed bivalves' resilience to the edge when both stressors were combined, given the high energetic costs required to simultaneously adapt to warmer seawater and lower pH (Lannig et al., 2010), thus compromising bivalve's ability to cope with TBBPA exposure.

To sum up, in a more generalized way, despite the different bioaccumulation and elimination patterns observed according to each compound, overall: i) warming (acting alone) seemed to play a key role for contaminants with longer half-lives, such Decs and TBBPA, enhancing their bioaccumulation; ii) acidification, on the other hand, seemed to particularly influence the bioaccumulation/elimination of ionisable compounds, such as TBBPA, iAs, PFOA and PFOS, impairing their bioaccumulation; iii) when both abiotic stressors are combined, these previously described effects are accentuated in the case of some contaminants(e.g. even higher Dec 602 bioaccumulation and even lower iAs bioaccumulation when both stressors are combined), but reversed for others (e.g. lower TBBPA and PFOS bioaccumulation in treatments simulating warming and acidification in isolation).

A direct link can also be established between the increased bioaccumulation and/or impaired detoxification of selected emerging contaminants, particularly Dec 602, 603, 604 and TBBPA under warmer temperatures and/or lower pH levels and the potential implications of these findings in seafood safety. Despite the presence of these compounds in seafood still remains unregulated, nor recommendations concerning tolerable limits of intake have been established due to the current lack of toxicological studies (e.g. EFSA, 2012), an adequate hazard assessment and risk analysis cannot be performed in the present study. Yet, the results suggest that human exposure to Decs and TBBPA through the consumption of seafood may increase under higher temperatures and lower pH. Such findings can be of particular concern, considering the fact that marine bivalves are important food resources from the economical and nutritional point of view, being among the most frequently consumed seafood species worldwide (FAO, 2016).

5. Conclusions

As clearly evidenced in this first study, temperature and pH can strongly affect the bioaccumulation and elimination patterns of emerging chemical contaminants in marine organisms, by affecting seawater physical and chemical properties, as well as animal metabolism and physiological responses. Overall, while warming in isolation enhanced the bioaccumulation of contaminants, particularly those with longer half-lives (e.g. Decs and TBBPA), acidification seemed to have a preponderant role on the bioaccumulation/elimination of ionisable compounds, reducing the bioaccumulation of TBBPA, iAs, PFOA and PFOS. Noteworthy, the combination of warming and acidification seemed to have reversed the effects promoted by both stressors acting alone, in the specific cases of TBBPA and PFOS (both ionisable), but not in the remaining compounds.

Despite variations were found according to the chemical compound, these results provide new insights on emerging contaminants' kinetics in bivalves maintained at higher temperatures and lower pH, and the potential human health risks associated to their consumption in the future. It also strengthens the need to carry out greater research efforts to understand how multiple environmental stressors interact with each other. Given the distinct behaviour of each contaminant and the different trends observed when warmer seawater and lower pH acted alone or in combination, future research should take into consideration regional trends (i.e. abiotic factors, pollution levels and diversity of contaminants) when addressing the expected effects of climate change on the kinetics of emerging contaminants, as the alterations of environmental conditions will certainly not affect marine ecosystems in the same way across the planet. Furthermore, because environmental stressors will unlikely occur in isolation, or all at once, different combinations of contaminant mixtures and abiotic conditions (exploring less pronounced to more severe scenarios) should be investigated to have a broader view of the toxicological impacts of climate change. Strengthening the knowledge on this matter will allow to incorporate the effects of climate change in future national and international regulations and set recommendations for human exposure to emerging contaminants, as well as to develop mitigation strategies to assure seafood safety in tomorrow's ocean.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.11.017.

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